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Smoking, but not Lipids, Lipoprotein (a) and Antibodies Against Oxidised LDL, is Correlated to the Expansion of Abdominal Aortic Aneurysms

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Objectives: *to study the role of smoking, lipids, lipoprotein (a), and autoantibodies against oxidised low density lipoprotein (Ab-oxLDL) in the expansion of small abdominal aortic aneurysms (AAA). To study the role of Ab-oxLDL and lp(a) in the progression of lower limb atherosclerosis.*
Methods and Materials: *one hundred and thirty-eight male patients with AAA were interviewed, examined, and their serum lipids and S-Ab-oxLDL determined. Of these, 117 were followed annually with ultrasound and underwent control scans and blood pressure measurements for a mean of 2.5 (range 1–5) years.*
Results: *initial AAA size, smoking and level of triglycerides were positively correlated to increased aneurysmal expansion, while beta-blocker medication was associated with decreased expansion. Besides initial AAA size, only smoking had persisting significance after adjustment of the other significant variables. Initial ankle brachial pressure index (ABI) and Lp(A) but not ab-oxLDL were significantly correlated to ABI change.*
Conclusion: *smoking cessation may inhibit aneurysmal expansion. Lipids seem to play a minor role in the progression of AAA.*

Key Words: Smoking; Lipids; Oxidized low density lipoprotein; Lipoprotein (a); Abdominal aortic aneurysm; Atherosclerosis; Progression.

Introduction

Predicting the expansion of small abdominal aortic aneurysms (AAA) is important for clinical management. Lipoprotein (A) (LpA) levels are related to atherosclerotic disease but the relationship between LpA-levels and the progression of AAA has not been analysed.[1]

Oxidized LDL is implicated in the initial damage of the vascular endothelium.[2–4] The concentration of antibodies against oxidised LDL (Ab-oxLDL) is elevated in patients with acute myocardial infarction (AMI)[2] and carotid stenosis.[2,3] The correlation was increased in cases with hypertriglyceridaemia.[5] The role of oxidised LDL in the pathogenesis of AAA is unknown.

The aim of the study was to relate AAA expansion with lipids, Lp(A) and Ab-oxLDL levels.

Material and Methods

Details of our AAA screening programme have been published previously.[6]

Patient sera were analysed for IgG antibodies against oxidised LDL by means of an enzyme-linked immunosorbent assay (ELISA) using oxLDL-coated microtitre plates, as previously described.[7,8]

P-Lipoprotein a (Lp(a)) was quantified with earlier reported and validated immunoradiometric assay (IRMA) (Pharmacia Diagnostics, Sweden).[9]

S-total-cholesterol, S-high density lipoprotein (HDL), and S-triglycerides were determined by commercial assays, while S-LDL was calculated as: S-cholesterol – S-HDL + (0.45*S-triglycerides).[10]

One hundred and seventy men had an AAA diagnosed at screening (4.0%).[11,12] Complete data were available in 138 cases (82%). Twenty-one were above 5 cm and referred for surgery, and the remaining 87% were offered annual ultrasound scans. Of these, 124 have now been followed for 1–5 years (average 2.5 years). Blood samples were complete in 117 (94%) of these patients. The mean expansion rate was mean 2.6 mm/year (SD:2.6). The ABI change was on average −2.2%/year (SD:6.8). The interobserver variations (def.: 2SD) of aortic diameter and ABI-change were 1.68 mm,[13] 0.16,[14] respectively. The intra-assay coefficients of variation of Ab-oxLDL and Lp(a) were 10% and 2.2%, respectively. The interassay coefficients

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of variation of Ab-oxLDL and Lp(a) were 25% and 3.1%, respectively.[9] The same assays were used for all the samples.

Also, Wilcoxon's rank sum test and Spearman's rank correlation analysis were used for the univariate analyses, while multiple linear regression analyses only were performed in significant univariate findings after securing that additivity, linearity, variance homogenity and normality of residuals were reasonable fulfilled. Unfortunately, lp(a) even after transformation failed to be reasonably normally distributed. The statistical software package used was SPSS 10.0.

Results

Table 1 shows the relationship between measured biochemical and clinical variables and use of beta-blockers, diet and smoking.

The relationship between lipids, Lp(a), Ab-oxLDL, AAA expansion, and ABI are tested upon beta-blocker medication, dietary habits, supplements of vitamin and Q10 (antioxidant) tablets, and smoking habits. Beta-blockers were associated with an adinose lipid profile, lower expansion and better ABI. However, these relationships disappeared after adjustment for smoking. Smoking was associated with aneurysmal expansion but not with change in ABI.

Besides the initial AAA size, S-triglycerides were correlated to expansion, while only the initial ABI and lipoprotein (A) were associated with the ABI-change-rate (Table 2). The initial AAA size remained significantly correlated to expansion after adjustment for smoking, while the correlation between triglycerides and expansion disappeared after adjustment for smoking.

Discussion

Only smoking and initial AAA size was correlated to expansion, while only initial ABI and concentration of lipoprotein (A) were correlated to ABI change rate.

Most reports studying the relation between aneurysmal size and expansion have also found initial AAA size of the AAA predictive for expansion[15–19] but we were not able to confirm earlier reports of increased expansion with increasing diastolic brachial blood pressure[20,21] or systolic ankle blood pressure.[16]

Smoking has frequently been reported to be associated with AAA in case control studies,[22–28] but apparently has only been reported once[29] to be correlated with expansion. This may be due to the association with the frequently coexisting atherosclerotic diseases, or due to the use of unreliable data obtained by interviews. The latter bias could be omitted by cotinine measurements.[23,29] Our smoking data was obtained from a questionnaire that was completed at home before the initial consultation. This may have limited the frequency of invalid data. Furthermore, smoking data was obtained at the following annual control scans; only one of the "non-smokers" started smoking. In spite of recommendations to stop smoking, unfortunately only one patient did so. This consistency of data suggests reliable data concerning smoking habits.

Beta-blockers were analysed because of earlier reports of lower expansion among beta-blocker-users.[21,30,31] The possible effect of beta-blockage is hypothesised to be caused by increased cross-linkage of collagen- and elastin.[32] Our observations confirm these previous observations. However, adjustment for smoking removed the significant associations, indicating that the apparently benefits of beta-blockage could be, at least partly, due to limited smoking among beta blocker users. This seems logical because acute myocardial infarction or other cardiac events are one of the most common reasons for beta-blockage. Such events could easily be more motivating to stop smoking than general health campaigns or an accidental finding of a asymptomatic small AAA. Furthermore, our earlier experience with a randomised intervention study with propranolol versus placebo could not show any effect. However, this was mainly because of a high frequency of side effects and decreased quality of life among those receiving propranolol, resulting in a low patient compliance in this study.[33]

A significant correlation between s-triglycerides and aneurysmal expansion was noticed. However, the correlation disappeared after adjustment for any of the other significant variables; beta-blockage, smoking, or initial AAA size. In spite of many earlier prospective studies of small AAA, only one study apart from our own seems to have reported increased expansion associated to hyperlipidaemia.[34] Furthermore, the association seems controversial; some reports have observed increased levels of various lipids in AAA-patients[22,35,36] while others have failed to do so.[23–25] These different reports may be explained by different patient populations with different frequencies of coexisting atherosclerotic diseases. Overall, the inconsistency of association in the present and previous studies suggests that lipids only seem to play a minor part in the progression of AAA, and thus indirectly in the pathogenesis of AAA.

Table 1. Beta blockage, dietary and smoking habits as dichotomous variables tested concerning antibodies against oxidised low density lipoprotein, lipids, lipoprotein (A), annual change in ankle-brachial-blood pressure index (ABI), and annual expansion rate of small abdominal aortic aneurysm. Median values with 25% and 75% quartile values shown in parentheses. *p*-Values of Wilcoxon's rank sum test.

Variable	Y N	S-Ab-oxLDL (units) Y/N	S-LDL (mmol/l) Y/N	S-HDL (mmol/l) Y/N	S-total cholesterol (mmol/l) Y/N	S-Lp(A) mmol/l	S-triglycerides (mmol/l) Y/N	ABI-change per year Y/N	Expansion rate (mm/year) Y/N
Beta-blockage	25 112	(17) 25 (39) (18) 21 (28) $p=0.22$	(0.9) 1.4 (1.6) (0.9) 1.1 (1.4) $p=0.22$	(0.9) 1.1 (1.2) (0.9) 1.1 (1.2) $p=0.93$	(5.8) 6.7 (7.1) (5.4) 6.0 (6.9) $p=0.13$	(2.5) 5.8 (31) (3.9) 10.3 (50) $p=0.97$	(2.3) 2.6 (3.1) (1.9) 2.4 (2.9) $p=0.15$	(1.0) 0.0 (−1.0) (1.0) −2.0 (−6.0) $p=0.01$	(1.0) 1.6 (2.7) (1.0) 2.5 (3.9) $p=0.01$
Regular eating habits	115 15	(18) 21 (30) (16) 21 (25) $p=0.33$	(0.9) 1.1 (1.4) (0.7) 1.0 (1.3) $p=0.24$	(0.9) 1.2 (1.4) (0.9) 1.1 (1.3) $p=0.29$	(5.5) 6.1 (7.0) (5.0) 6.0 (6.9) $p=0.70$	(3.0) 9.8 (29) (4.7) 8.5 (13) $p=0.73$	(1.9) 2.4 (2.9) (2.4) 2.6 (3.4) $p=0.24$	(1.0) −1.0 (−5.0) (1.5) 0.5 (−4.0) $p=0.22$	(1.0) 1.9 (3.2) (1.6) 2.8 (4.6) $p=0.09$
Tablet supply of vitamins	64 67	(18) 21 (28) (17) 22 (30) $p=0.85$	(0.9) 1.1 (1.4) (0.9) 1.1 (1.4) $p=0.95$	(1.0) 1.2 (1.3) (0.9) 1.2 (1.4) $p=0.68$	(5.4) 6.0 (7.0) (5.7) 6.2 (6.6) $p=0.97$	(3.7) 8.0 (29) (3.6) 10.5 (28) $p=0.63$	(2.0) 2.5 (3.0) (1.8) 2.4 (3.0) $p=0.55$	(1.0) −1.0 (−5.0) (1.0) −2.0 (−5.0) $p=0.88$	(1.1) 2.0 (3.1) (0.9) 1.9 (3.7) $p=0.58$
Tablet supply of Q10	12 110	(20) 21 (23) (17) 21 (28) $p=0.86$	(1.0) 1.2 (1.3) (0.9) 1.1 (1.5) P-0.90	(0.9) 1.2 (1.4) (0.9) 1.2 (1.3) $p=0.93$	(5.7) 6.1 (6.7) (5.5) 6.1 (7.0) $p=0.95$	(0.0) 2.5 (21) (4.1) 10.5 (29) $p=0.04$	(1.9) 2.4 (2.9) (1.9) 2.4 (2.9) $p=0.89$	(1.0) 0.5 (−1.0) (1.0) −2.0 (−6.0) $p=0.04$	(0.9) 2.0 (2.4) (1.0) 2.5 (3.9) $p=0.19$
Smoking	82 52	(17) 21 (29) (18) 22 (28) $p=0.79$	(0.8) 1.1 (1.4) (1.0) 1.1 (1.5) $p=0.33$	(0.9) 1.2 (1.3) (1.0) 1.2 (1.4) $p=0.87$	(5.5) 6.0 (6.5) (5.4) 6.3 (7.1) $p=0.26$	(2.8) 9.9 (28) (4.1) 10.1 (32) $p=0.66$	(1.9) 2.3 (2.8) (2.0) 2.5 (3.2) $p=0.36$	(1.0) −1.0 (−6.0) (0.0) −2.0 (−4.0) $p=0.42$	(1.0) 2.6 (4.1) (0.9) 1.7 (2.8) $p=0.01$*

*: $p<0.05$ (p-value of multivariate regression analysis adjusting for smoking). Only performed on univariate significant tests.

Table 2. Spearman's non parametric correlation coefficients of selected continuous variables focusing on aneurysmal expansion rate and rate of decreasing ankle blood pressure index (ABI) as dependent variables.

	AAA-size (mm)	Initial ABI (%)	ABI-change (%/year)	S-LDL (mmol/l)	S-HDL (mmol/l)	S-tri-glycerides (mmol/l)	S-total cholesterol (mmol/l)	Ab-oxLDL (units/l)	Lp(a) (mmol/l)	Body mass index (kg/w^2)
Initial AAA-size (mm)	1.00	0.11 (0.21)	0.11 (0.73)	0.013 (0.99)	0.10 (0.32)	0.01 (0.92)	0.01 (0.90)	−0.16 (0.08)	−0.04 (0.74)	0.03 (0.75)
AAA-expansion (mm/year)	0.30 *(0.01)**	0.06 (0.50)	0.11 (0.20)	0.031 (0.97)	0.01 (0.91)	0.21* (0.04)	0.17 (0.14)	−0.15 (0.09)	0.11 (0.33)	−0.01 (0.94)
Initial ABI	0.11 (0.21)	1.00	0.33 *(0.01)**	−0.01 (0.98)	0.01 (0.89)	−0.07 (0.51)	−0.09 (0.43)	−0.12 (0.20)	−0.18 (0.06)	0.09 (0.30)
ABI-change (per cent/year)	0.11 (0.73)	0.33 *(0.01)**	1.00	0.01 (0.98)	0.01 (0.91)	−0.11 (0.31)	−0.04 (0.73)	−0.15 (0.11)	0.26 *(0.02)*	0.04 (0.64)

ABI: ankle brachial systolic blood pressure index.
HDL: High density lipoprotein.
LDL: Low density lipoprotein.
Ab-oxLDL: antibodies angainst oxidised LDL.
Lp(a): lipoprotein A.
*: $p<0.05$ after multiple linear regression analysis adjusting for smoking. (Only performed on univariate significant tests, and log(lp(a)) concerning lp(a).

The concentration of antibodies against malondialdehyde-modified LDL has been shown to be significantly elevated in patients with AMI and carotid-stenosis, but not in angina pectoris,[37] and positively correlated to the progression of carotid stenosis. This correlation was increased by presence of hypertriglyceridaemia.[5] However, the level of ab-oxLDL could not be associated to aneurysmal progression. This is hardly due to a large variation of measurement because only one scanner and two observers were used. Validation of our measurements have earlier been reported;[13] 95% of their measurements are within 2 mm difference.[14] Furthermore, the mean average expansion rate was 2.6 mm/year, and the average observation time was 2.5 years. Consequently, a clinical significant correlation to expansion must be expected to be detectable. Also, Ab-oxLDL were not associated with a faster decrease in ABI. However, the ABI changes were relatively minor compared with expansion rates, and the variation of the ABI measurements was larger (15% of the measurements are measured within 15% difference).

The negative results concerning ab-oxLDL cannot be taken as a sign of minor pathogenetic importance of oxidised LDL because plasma oxidised LDL seems to increase the transposition through the arterial endothelium and wall, and to increase the degradation of the wall.[38] Consequently, oxidised LDL could play a part in the aneurysmal and atherosclerotic process. The negative results concerning Ab-oxLDL may rather question whether the antibodies will reflect the degree of this transposition because oxidized LDL seems only present in the circulation for minutes.[38]

Finally, lipoprotein (A) levels have earlier been correlated to the incidence of intermittent claudication.[1] By adding objective and prospective measurements, our results supports this, while the lack of any correlation to aneurysmal expansion indicates partly different pathogenetic factors between atherosclerosis and aneurysmal degradation.

Conclusion

If anything, lipids seem to play only a minor role in the progression of AAA, and pharmacological intervention to lower the serum levels can only be justified in order to prevent atherosclerotic events. In contrast, smoking cessation may inhibit aneurysmal expansion. Antibodies against oxidised LDL seem not to be predictive of aneurysmal or atherosclerotic progression, and can at this point not be recommended for monitoring or decision-making concerning antioxidant therapy. However, the role of oxidised low density lipoprotein remains unsolved.

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